

=> S ENTEROKINASE/CN\
 'CN\' IS NOT A VALID FIELD CODE
 L1 0 ENTEROKINASE/CN\
 => S ENTEROKINASE/CN
 L2 1 ENTEROKINASE/CN

=> S STREPTAVIDIN/CN
 L3 1 STREPTAVIDIN/CN

FILE 'CAPLUS' ENTERED AT 13:52:37 ON 03 JUN 2003

=> S L2;S L3
 L4 614 L2

L5 3477 L3

=> S ENTEROKINASE OR L2;S STREPTAVIDIN OR L3
 983 ENTEROKINASE
 6 ENTEROKINASES
 983 ENTEROKINASE
 (ENTEROKINASE OR ENTEROKINASES)
 614 L2
 L6 1080 ENTEROKINASE OR L2

6578 STREPTAVIDIN
 24 STREPTAVIDINS
 6580 STREPTAVIDIN
 (STREPTAVIDIN OR STREPTAVIDINS)
 3477 L3
 L7 6591 STREPTAVIDIN OR L3

=> S RECOGNITION;S CLEAVABLE OR CLEAVE OR CLEAVES
 87877 RECOGNITION
 143 RECOGNITIONS
 L8 87945 RECOGNITION
 (RECOGNITION OR RECOGNITIONS)

3710 CLEAVABLE
 1 CLEAVABLES
 3711 CLEAVABLE
 (CLEAVABLE OR CLEAVABLES)
 8621 CLEAVE
 6972 CLEAVES
 14906 CLEAVE
 (CLEAVE OR CLEAVES)
 6972 CLEAVES
 L9 18506 CLEAVABLE OR CLEAVE OR CLEAVES

=> S L6 AND L7
 L10 7 L6 AND L7

=> D 1-7 CBIB ABS

L10 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2003 ACS

2002:965025 Document No. 138:35722 ***Enterokinase*** cleavage sequences
 useful for isolation of fusion proteins. Ley, Arthur Charles; Luneau,
 Christopher Jon; Ladner, Robert Charles (USA). U.S. Pat. Appl. Publ. US
 2002192789 A1 20021219, 67 pp., Cont.-in-part of U. S. Ser. No. 597,321,
 abandoned. (English). CODEN: USXXCO. APPLICATION: US 2001-884767
 20010619. PRIORITY: US 2000-597321 20000619.

AB Novel ***enterokinase*** cleavage sequences are provided. To identify
 novel ***enterokinase*** cleavage sequences, a substrate phage
 library, having a diversity of about 2 .times. 10⁸ amino acid sequences,
 was screened against ***enterokinase***. The substrate phage library
 was design to include a peptide-variegated region in the display

polypeptide consisting of 13 consecutive amino acids and allowing any amino acid residue except cysteine to occur at each position. The substrate phage library was also characterized by inclusion of an N-terminal tandem arrangement of a linear and a disulfide-constrained ***streptavidin*** recognition sequence. The screen was carried through a total of 5 rounds of increasing stringency to obtain phage that could be released by incubation with recombinant light chain ***enterokinase*** after binding to immobilized ***streptavidin***. Also disclosed are methods for the rapid isolation of a protein of interest present in a fusion protein construct including a novel ***enterokinase*** cleavage sequence of the present invention and a ligand recognition sequence for capturing the fusion construct on a solid substrate. Preferred peptides of the present invention (e.g., Asp-Ile-Asn-Asp-Asp-Arg-Xaa) show rates of cleavage (kcat/Km) up to 30-fold that of the known ***enterokinase*** cleavage substrate (Asp)4-Lys-Ile.

L10 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2003 ACS

2002:555369 Document No. 137:124189 Vaccine compositions comprising molecular antigen array against cancer, infection, and allergy. Renner, Wolfgang A.; Bachmann, Martin; Tissot, Alain; Maurer, Patrick; Lechner, Franziska; Sebbel, Peter; Piossek, Christine (Cytos Biotechnology A.-G., Switz.). PCT Int. Appl. WO 2002056905 A2 20020725, 442 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-IB166 20020121. PRIORITY: US 2001-PV262379 20010119; US 2001-PV288549 20010504; US 2001-PV326998 20011005; US 2001-PV331045 20011107.

AB The present invention is related to the fields of mol. biol., virol., immunol. and medicine. The invention provides a compn. comprising an ordered and repetitive antigen or antigenic determinant array. The invention also provides a process for producing an antigen or antigenic determinant in an ordered and repetitive array. The ordered and repetitive antigen or antigenic determinant is useful in the prodn. of vaccines for the treatment of infectious diseases, the treatment of allergies and as a pharmaccine to prevent or cure cancer and to efficiently induce self-specific immune responses, in particular antibody responses.

L10 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2003 ACS

2001:935666 Document No. 136:66211 Novel ***enterokinase*** cleavage sequences and their use in protein isolation and purification. Ley, Arthur Charles; Luneau, Christopher Jon; Ladner, Robert Charles (Dyax Corp., USA). PCT Int. Appl. WO 2001098366 A2 20011227, 119 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US19539 20010619. PRIORITY: US 2000-597321 20000619.

AB Novel ***enterokinase*** cleavage sequences are provided. Also disclosed are methods for the rapid isolation of a protein of interest present in a fusion protein construct including a novel ***enterokinase*** cleavage sequence of the present invention and a ligand recognition sequence for capturing the fusion construct on a solid substrate. Thus, using phage display technol., a no. of novel ***enterokinase*** recognition sequences were discovered that provide a highly specific substrate for rapid cleavage by ***enterokinase***. These show rates of cleavage up to thirty times that of the known ***enterokinase*** cleavage substrate (Asp)4-Lys-Ile.

L10 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2003 ACS

2000:688462 Document No. 133:265653 Protein isolation and analysis. Carr,

Francis J. (Biovation Limited, UK). PCT Int. Appl. WO 2000057183 A1
20000928, 53 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA,
BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB,
GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU,
SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH,
CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE,
NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO
2000-GB1015 20000317. PRIORITY: GB 1999-6551 19990323; GB 1999-7057
19990329; GB 1999-7641 19990406; GB 1999-14874 19990628; GB 1999-15363
19990702; GB 1999-15677 19990706; GB 1999-16511 19990714; GB 1999-20503
19990831; GB 1999-22285 19990921.

AB Novel methods for the identification and/or sequencing of proteins are
provided. These methods are particularly suited to screening antibody
libraries and in preferred embodiments make use of mass spectrometry
techniques for direct or indirect sequencing.

L10 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2003 ACS

2000:587045 Document No. 133:173000 Fusion proteins of carriers and random
peptide libraries and their use in screening for receptor ligands. Hart,
Charles P. (Affymax Technologies N.V., Neth.). U.S. US 6107059 A
20000822, 39 pp. (English). CODEN: USXXAM. APPLICATION: US 1992-876288
19920429.

AB A random peptide library constructed by transforming host cells with a
collection of recombinant vectors that encode a fusion protein comprised
of a carrier protein fused to a random peptide through a proteolytic
cleavage site can be used to identify ligands that bind to a receptor.
The screening method results in the formation of a complex comprising the
fusion protein bound to a receptor through the random peptide ligand, and
the random peptide can easily be identified and analyzed by virtue of the
carrier protein and assocd. proteolytic cleavage site.

L10 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2003 ACS

1998:106052 Document No. 128:163644 A homogeneous nucleic acid detection
method utilizing simultaneous target and signal amplification. Hepp,
Jozsef; Lengyel, Zsolt; Pande, Rajiv; Botyanszki, Janos; Sahin-Toth,
Miklos (Navix, Inc., USA; Hepp, Jozsef; Lengyel, Zsolt; Pande, Rajiv;
Botyanszki, Janos; Sahin-Toth, Miklos). PCT Int. Appl. WO 9804739 A2
19980205, 73 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG,
BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO,
NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US,
UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ,
CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML,
MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION:
WO 1997-US12415 19970716. PRIORITY: US 1996-692825 19960725.

AB A method for detg. the presence of a target nucleic acid in a sample using
a two-stage target cycling reaction is described. The method uses a
hybridization probe that is complexed with an activator. When the probe
hybridizes with its target the activator is released. The activator then
interacts with an analog of the target sequence that is immobilized via an
anchor moiety, leading to its release and the generation of a signal
specific to the released cleavage products. The released target analog
then restarts the cyclic reaction by binding to a second probe, which
effectuates release of a second activator, and so on. This cyclic
reaction amplifies the signal generated from a single target nucleic acid
mol. in the sample, which greatly enhances the level of target detection
that can be expected. The analog and the target have to be sepd. in the
assay and this can be brought about by immobilizing them on sep. surfaces
or using a membrane that is permeable to the released activator and target
analog, but not the target or the free probe. An enzyme-based version of
the assay is demonstrated. An oligonucleotide probe with a central
oligoribonucleotide was prepd., conjugated with ***enterokinase***,
and immobilized. The anchored target analog was conjugated to
trypsinogen. When the target sequence hybridizes to the
enterokinase labeled probe, it is cleaved at the RNA moiety with
RNase H to release the ***enterokinase***. The released
enterokinase cleaves the trypsinogen to release the
oligonucleotide. The resulting trypsin can be assayed with a chromogenic
substrate.

streptavidin fusion proteins with recombinant microorganisms.
Kopetzki, Erhard (Boehringer Mannheim GmbH, Germany; Kopetzki, Erhard).
PCT Int. Appl. WO 9718314 A1 19970522, 38 pp. DESIGNATED STATES: W: AL,
AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES,
FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM;
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR,
IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (German). CODEN:
PIXXD2. APPLICATION: WO 1996-EP4850 19961106. PRIORITY: DE 1995-19542702
19951116.

AB The invention relates to a process for recombinant prepn. of peptides by
expression of a DNA in micro-organisms, which DNA codes for a fusion
protein made of ***streptavidin*** and one of the said peptides.
Streptavidin and the peptide are bound by a peptide sequence which
can be cleaved by an endoprotease. The process also includes isolation
of the insol., inactive protein, solubilization of the inactive protein
using a denaturant, diln. of the denaturant at a pH value of between 8.5
and 11 until cleaving of the fusion protein by an endoprotease can take
place, cleaving of the fusion protein, lowering of the pH value until
streptavidin and non-cleaved fusion protein ppt., and cleaning of
the desired peptide from the supernatant. Said process is particularly
suitable for producing parathormone and urodilatin and fragments thereof.

=> S FUSION

214978 FUSION

8009 FUSIONS

L11 219318 FUSION

(FUSION OR FUSIONS)

=> D HIS

(FILE 'HOME' ENTERED AT 13:50:58 ON 03 JUN 2003)

FILE 'REGISTRY' ENTERED AT 13:51:07 ON 03 JUN 2003

L1 0 S ENTEROKINASE/CN\
L2 1 S ENTEROKINASE/CN
L3 1 S STREPTAVIDIN/CN

FILE 'CAPLUS' ENTERED AT 13:52:37 ON 03 JUN 2003

L4 614 S L2
L5 3477 S L3
L6 1080 S ENTEROKINASE OR L2
L7 6591 S STREPTAVIDIN OR L3
L8 87945 S RECOGNITION
L9 18506 S CLEAVABLE OR CLEAVE OR CLEAVES
L10 7 S L6 AND L7
L11 219318 S FUSION

=> S L11 AND L6

L12 216 L11 AND L6

=> S L6(10A) (L8 OR L9)

L13 36 L6(10A) (L8 OR L9)

=> S L13 AND L11

L14 30 L13 AND L11

=> D 1-30 CBIB ABS

2003:154610 Document No. 138:199942 Method for extracellular recombinant
production of a target protein (such as .beta.-endorphin) employing
Escherichia coli OmpF and expression vector pOmpF6. Lee, Sang-Yup; Jeong,
Ki-Jun (Korea Advanced Institute of Science and Technology, S. Korea).
PCT Int. Appl. WO 2003016538 A1 20030227, 32 pp. DESIGNATED STATES: W:
CN, JP, US. (English). CODEN: PIXXD2. APPLICATION: WO 2002-KR1547

AB The invention discloses the expression vector pOmpF6, which contains the ampicillin-resistance gene, the Escherichia coli outer membrane protein OmpF gene, and the OmpF promoter. The invention also discloses E. coli transformed with pOmpF6, and methods for extracellular prodn. of target proteins employing transformed microorganisms and expression vector pOmpF6. The invention further discloses a recombinant expression vector constructed using pOmpF6, into which a gene encoding a target protein and a gene encoding an oligopeptide for cleavage by proteolytic enzyme have been inserted, resulting in a OmpF-oligopeptide-target ***fusion*** protein. The invention relates that the said OmpF- ***fusion*** protein can be secreted efficiently into culture media of recombinant E. coli transformed with said vector, and that the desired target protein can be removed from OmpF by enzymic cleavage. Specifically, the invention provides the recombinant expression vector pOmpF6.beta.E encoding OmpF-oligopeptide-.beta.-endorphin, wherein said oligopeptide is recognized by Factor Xa. The invention also demonstrated the use of said pOmpF6.beta.E in recombinant prodn. of .beta.-endorphin in transformed E. coli.

L14 ANSWER 2 OF 30 CAPLUS COPYRIGHT 2003 ACS

2002:965025 Document No. 138:35722 Enterokinase cleavage sequences useful for isolation of ***fusion*** proteins. Ley, Arthur Charles; Luneau, Christopher Jon; Ladner, Robert Charles (USA). U.S. Pat. Appl. Publ. US 2002192789 A1 20021219, 67 pp., Cont.-in-part of U. S. Ser. No. 597,321, abandoned. (English). CODEN: USXXCO. APPLICATION: US 2001-884767 20010619. PRIORITY: US 2000-597321 20000619.

AB Novel enterokinase cleavage sequences are provided. To identify novel enterokinase cleavage sequences, a substrate phage library, having a diversity of about 2 .times. 10⁸ amino acid sequences, was screened against enterokinase. The substrate phage library was design to include a peptide-variegated region in the display polypeptide consisting of 13 consecutive amino acids and allowing any amino acid residue except cysteine to occur at each position. The substrate phage library was also characterized by inclusion of an N-terminal tandem arrangement of a linear and a disulfide-constrained streptavidin recognition sequence. The screen was carried through a total of 5 rounds of increasing stringency to obtain phage that could be released by incubation with recombinant light chain enterokinase after binding to immobilized streptavidin. Also disclosed are methods for the rapid isolation of a protein of interest present in a ***fusion*** protein construct including a novel ***enterokinase*** cleavage sequence of the present invention and a ligand ***recognition*** sequence for capturing the ***fusion*** construct on a solid substrate. Preferred peptides of the present invention (e.g., Asp-Ile-Asn-Asp-Asp-Arg-Xaa) show rates of cleavage (kcat/Km) up to 30-fold that of the known enterokinase cleavage substrate (Asp)4-Lys-Ile.

L14 ANSWER 3 OF 30 CAPLUS COPYRIGHT 2003 ACS

2002:900267 Document No. 138:168852 The calcium-binding protein of Entamoeba histolytica as a ***fusion*** partner for expression of peptides in Escherichia coli. Reddi, Honey; Bhattacharya, Alok; Kumar, Vijay (Virology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, 110 067, India). Biotechnology and Applied Biochemistry, 36(3), 213-218 (English) 2002. CODEN: BABIEC. ISSN: 0885-4513. Publisher: Portland Press Ltd..

AB We describe the construction of an Escherichia coli expression vector, CBP that allows the C-terminal ***fusion*** of heterologous proteins to the calcium-binding protein (CaBP) of the parasitic protozoan Entamoeba histolytica. The intrinsic nature of this protein to remain sol. on heat treatment has been exploited in its use as a novel ***fusion*** partner. The presence of a histidine tag and an ***enterokinase*** ***recognition*** site, aid in the affinity purifn. and proteolytic cleavage of the ***fusion*** protein. The efficacy of the vector was tested using the preS1 region of the envelope protein of the hepatitis B virus. The CaBP-preS1 ***fusion*** protein partitioned in the sol. fraction on heat treatment and this facilitated its rapid purifn.

L14 ANSWER 4 OF 30 CAPLUS COPYRIGHT 2003 ACS

2002:752376 Document No. 137:277775 Protein and cDNA of human cancer cell-specific HLA-F antigen and uses in cancer diagnosis. Egawa, Kohji (Medinet Co., Ltd., Japan; Kimura, Yoshiji). Eur. Pat. Appl. EP 1245675

A1 2021002, 20 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR. (English). CODEN: EPXXDW. APPLICATION: EP 2001-400804 20010328.

AB The invention provides protein and cDNA of human cancer cell-specific HLA-F antigen isolated from U937 cells. This invention provides a method of detecting cancer cells in any organ and irresp. of causes of the tumors. In said method, a new antigenic substance that cancer cells commonly produce in a cancer cell-specific manner is first identified and, then, an antibody produced in response to this antigen is detected in body fluid of cancer patients. Specifically, this is achieved by detecting the anti-HLA-F antibody specific to the cancer cell-specific HLA-F antigen coded by the HLA-F gene.

L14 ANSWER 5 OF 30 CAPLUS COPYRIGHT 2003 ACS

2002:556192 Document No. 137:348212 Expression, purification, and characterization of a biologically active bovine enterokinase catalytic subunit in Escherichia coli. Yuan, Liu-Di; Hua, Zi-Chun (College of Life Sciences, State Key Laboratory of Pharmaceutical Biotechnology and Institute of Molecular and Cell Biology, Department of Biochemistry, Nanjing University, Nanjing, 210093, Peop. Rep. China). Protein Expression and Purification, 25(2), 300-304 (English) 2002. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB Enterokinase (EC 3.4.21.9) is a serine proteinase in the duodenum that exhibits specificity for the sequence (Asp)4-Lys. It converts trypsinogen to trypsin. Its high specificity for the ***recognition*** site makes ***enterokinase*** (EK) a useful tool for in vitro cleavage of ***fusion*** proteins. CDNA encoding the catalytic chain of Chinese bovine enterokinase was cloned and its encoding amino acid sequence is identical to the previously reported sequence although there are two one-base mutations which do not change the encoded amino acid. The EK catalytic subunit cDNA was cloned into plasmid pET32a, and fused downstream to the ***fusion*** partner thioredoxin (Trx) and the following DDDDK ***enterokinase*** ***recognition*** sequence. The recombinant bovine enterokinase catalytic subunit was expressed in Escherichia coli BL21(DE3), and most products existed in sol. form. After an in vivo autocatalytic cleavage of the recombinant Trx-EK catalytic domain ***fusion*** protein, intact, biol. active EK catalytic subunit was released from the ***fusion*** protein. The recombinant intact EK catalytic subunit was purified to homogeneity with a specific activity of 720 AUs/mg protein through ammonium sulfate pptn., DEAE chromatog., and gel filtration. The purified intact EK catalytic subunit has a Km of 0.17 mM, and Kcat is 20.8 s⁻¹. From 100 mL flask culture, 4.3 mg pure active EK catalytic subunits were obtained.

L14 ANSWER 6 OF 30 CAPLUS COPYRIGHT 2003 ACS

2002:533982 Document No. 137:104772 Zymogen expression-activation system for preparation of serine protease catalytic domains for use in skin care compositions. Darrow, Andrew; Qi, Jenson; Andrade-Grodon, Patricia (Ortho-Mcneil Pharmaceutical, Inc., USA). U.S. US 6420157 B1 20020716, 74 pp., Cont.-in-part of U. S. Ser. No. 303,162. (English). CODEN: USXXAM. APPLICATION: US 1999-386642 19990831. PRIORITY: US 1999-303162 19990430.

AB An expression vector comprising a pre sequence, a pro sequence, an affinity tag sequence, a cloning site for in frame insertion of a protease catalytic domain-encoding cassette and a method for producing a zymogen with recombinant cells contg. the vector is disclosed. The protease catalytic domain ***fusion*** protein produced by the recombinant cells as well as its use in a topical skin care compn. are further disclosed. Compds. which modulate the activity of the protease may be identified by incubating the protease protein with a labeled substrate and measuring a change in the labeled substrate. Thus, an expression construct encoding a ***fusion*** protein comprising the chymotrypsinogen prepeptide fused to a FLAG peptide fused to an ***enterokinase*** - ***cleavable*** propeptide fused to human prostasin catalytic domain fused to a hexahistidine tag was expressed in Drosophila Sf9 cells. The prostasin ***fusion*** protein was added to Ni-NTA agarose beads and the bound zymogen was activated by digestion with enterokinase.

L14 ANSWER 7 OF 30 CAPLUS COPYRIGHT 2003 ACS

2002:10642 Document No. 136:81960 Cloning and sequencing of Fucus distichus vanadium bromoperoxidase and construction and uses of recombinant minimal

catalytic vanadiumhaloperoxidase. Vreeland, Valerie (Regents of the University of California, USA). PCT Int. Appl. WO 2002000838 A2 20020103, 56 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US18602 20010607. PRIORITY: US 2000-596794 20000619.

AB Cloning and sequencing of *Fucus distichus* vanadium bromoperoxidase is reported. The amino acid sequence and the encoding cDNA sequence of the *F. distichus* enzyme are disclosed. Identification of the minimal catalytic unit of the *F. distichus* vanadium haloperoxidase is described. The present invention provides new recombinantly produced minimal catalytic vanadium haloperoxidases from *F. distichus*. The enzymes are useful in a no. of industrial applications.

L14 ANSWER 8 OF 30 CAPLUS COPYRIGHT 2003 ACS

2001:935666 Document No. 136:66211 Novel enterokinase cleavage sequences and their use in protein isolation and purification. Ley, Arthur Charles; Luneau, Christopher Jon; Ladner, Robert Charles (Dyax Corp., USA). PCT Int. Appl. WO 2001098366 A2 20011227, 119 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US19539 20010619. PRIORITY: US 2000-597321 20000619.

AB Novel enterokinase cleavage sequences are provided. Also disclosed are methods for the rapid isolation of a protein of interest present in a ***fusion*** protein construct including a novel ***enterokinase*** cleavage sequence of the present invention and a ligand ***recognition*** sequence for capturing the ***fusion*** construct on a solid substrate. Thus, using phage display technol., a no. of novel ***enterokinase*** ***recognition*** sequences were discovered that provide a highly specific substrate for rapid cleavage by enterokinase. These show rates of cleavage up to thirty times that of the known enterokinase cleavage substrate (Asp)4-Lys-Ile.

L14 ANSWER 9 OF 30 CAPLUS COPYRIGHT 2003 ACS

2001:897218 Document No. 136:364426 Recombinant enterokinase light chain with affinity tag: expression from *Saccharomyces cerevisiae* and its utilities in ***fusion*** protein technology. Choi, Seong, II; Song, Hye Won; Moon, Jae Woong; Seong, Baik L. (Department of Biotechnology, College of Engineering and Bioproducts Research Center, Yonsei University, Seoul, 120-749, S. Korea). Biotechnology and Bioengineering, 75(6), 718-724 (English) 2001. CODEN: BIBIAU. ISSN: 0006-3592. Publisher: John Wiley & Sons, Inc..

AB Enterokinase and recombinant ***enterokinase*** light chain (rEKL) have been used widely to ***cleave*** ***fusion*** proteins with the target sequence of (Asp)4-Lys. In this work, we show that their utility as a site-specific cleavage agent is compromised by sporadic cleavage at other sites, albeit at low levels. Further degrdn. of the ***fusion*** protein in cleavage reaction is due to an intrinsic broad specificity of the enzyme rather than to the presence of contaminating proteases. To offer facilitated purifn. from fermn. broth and efficient removal of rEKL after cleavage reaction, thus minimizing unwanted cleavage of target protein, histidine affinity tag was introduced into rEKL. Utilizing the secretion enhancer peptide derived from the human interleukin 1.beta., the recombinant EKL was expressed in *Saccharomyces cerevisiae* and efficiently secreted into culture medium. The C-terminal His-tagged EKL was purified in a single-step procedure on nickel affinity chromatog. It retained full enzymic activity similar to that of EKL, whereas the N-terminal His-tagged EKL was neither efficiently purified nor had any enzymic activity. After cleavage reaction of ***fusion*** protein, the C-terminal His-tagged EKL was efficiently removed from the

reaction mixt. by a single passage through nickel-NTA spin column. The simple affinity tag renders rEKL extremely useful for purifn., post-cleavage removal, recovery, and recycling and will broaden the utility and the versatility of the enterokinase for the prodn. of recombinant proteins.

L14 ANSWER 10 OF 30 CAPLUS COPYRIGHT 2003 ACS

2001:284120 Document No. 134:307589 Purification of recombinant proteins fused to multiple epitopes. Brizzard, Bill; Hernan, Ron (Sigma-Aldrich Co., USA). PCT Int. Appl. WO 2001027293 A1 20010419, 51 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US25693 20000920. PRIORITY: US 1999-415000 19991008.

AB The present invention provides novel identification polypeptides contg. multiple copies of an antigenic domain joined in tandem to provide increased sensitivity for the detection and purifn. of target peptides, a cleavable linking sequence and optionally a spacer domain. Further provided are hybrid polypeptide mols. composed of an identification polypeptide and a target peptide which are produced by recombinant DNA technol. and purified using affinity chromatog. using one or more ligands. Accordingly, also provided are DNA expression vectors contg. DNA encoding for identification polypeptides and methods for using such identification polypeptides for the purifn. of target peptides. Thus, a vector was constructed for expression of proteins in mammalian host cells using a modified version of the FLAG expression system, which contains three FLAG sequences in tandem. The first two flag peptides are modified sequences (Asp-Tyr-Lys-Asp-His-Asp) with either a Gly or Ile spacer domain between the two sequences. Proteolytic ***recognition*** sites are also included for cleavage by ***enterokinase***, thrombin, or factor Xa. The p3XFLAG-CMV-7 expression vector contains the human cytomegalovirus promoter region necessary for constitutive expression of cloned genes in many mammalian cell lines, as well as the Kozak consensus sequence, a multiple cloning site, and the SV40 origin of replication. A 10-fold increase in detection limit of the triple FLAG-bacterial alk. phosphatase was obsd. compared to the single FLAG-BAP ***fusion*** protein, and 500 pg of purified 2XFlag bacterial alk. phosphatase could be detected with exposures as short as 1 min. A 10-fold increase detection was also demonstrated in both dot blot and ELISA assay.

L14 ANSWER 11 OF 30 CAPLUS COPYRIGHT 2003 ACS

2000:790611 Document No. 133:330529 Expression systems for the secretory manufacture of serine proteinases involving accurate processing of a zymogen. Darrow, Andrew; Qi, Jensen; Andrade-Gordon, Patricia (Ortho-McNeil Pharmaceutical Research Inc., USA). PCT Int. Appl. WO 2000066709 A2 20001109, 89 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US9973 20000413. PRIORITY: US 1999-303162 19990430.

AB We describe the DNA sequences encoding an expression vector system that will permit, through limited proteolysis, the activation of expressed zymogen precursor of (S1) serine proteases in a highly controlled and reproducible fashion. The processed expressed protein, once activated, is rendered in a form amenable to measuring the catalytic activity. This catalytic activity of the activated form, is often a more accurate representation of the mature S1 protease gene product relative to the unprocessed zymogen precursor. Thus, this series of zymogen activation constructs represents a significant system for the anal. and characterization of serine protease gene products. Construction of analogs of a no. of proteinases that allowed them to be processed with enterokinase is demonstrated. The signal peptide of bovine prolactin is

used to direct secretion.

L14 ANSWER 12 OF 30 CAPLUS COPYRIGHT 2003 ACS

2000:615648 Document No. 134:13775 Expression of a Kallikrein-like Protease from the Snake Venom: Engineering of Autocatalytic Site in the ***Fusion*** Protein to Facilitate Protein Refolding. Hung, Chin-Chun; Chiou, Shyh-Horng (Institute of Biochemical Sciences, Coll. Sci., Natl. Taiwan Univ., Taiwan). Biochemical and Biophysical Research Communications, 275(3), 924-930 (English) 2000. CODEN: BBRCA9. ISSN: 0006-291X. Publisher: Academic Press.

AB In order to circumvent the difficulty encountered in the expression and purifn. of the recombinant products in E. coli system, we have developed a novel and facile method of removing the polyhistidine tag from target proteins after heterologous gene expression. The expression of a serine protease (Tm-5) from Taiwan habu (Trimeresurus mucrosquamatus) is taken as an exemplar to illustrate the basic rationales and protocols involved. In place of an ***enterokinase*** ***recognition*** site, a polyhistidine tag linked to an autocatalyzed site based on cleavage specificity of the serine protease flanking on the 5'-end of Tm-5 clone sequence was engineered before protein expression in E. coli system. Renaturation of the ***fusion*** protein after expression revealed that the recombinant protease had refolded successfully from the inclusion bodies. Upon autocleavage of the expressed protease, the polyhistidine tag with addnl. amino acid residues appended to the N-terminus of the coding sequence is found to be removed accordingly. The protein expressed and purified by this new strategy possesses a mol. wt. of approx. 28,000 in accord with the expected value for this venom protease. Further characterization of the recombinant protein employing a variety of techniques which include immunoblot anal., RP-HPLC, ESI-MS, and N-terminal amino acid sequencing all shows indistinguishable properties to those of the isolated native protease. Most noteworthy is that the recombinant Tm-5 protease also exhibits amidase activity against N-benzoyl-Pro-Phe-Arg-p-nitroanilide, a unique and strict substrate for native Tm proteases reported previously. (c) 2000 Academic Press.

L14 ANSWER 13 OF 30 CAPLUS COPYRIGHT 2003 ACS

2000:592843 Document No. 133:182929 ***Fusion*** proteins of the collagen-binding fragment of human fibronectin ligated to physiologically active polypeptides. Ishikawa, Tetsuya; Kitajima, Takashi (Terumo Kabushiki Kaisha, Japan). PCT Int. Appl. WO 2000049159 A1 20000824, 135 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-JP964 20000221. PRIORITY: JP 1999-41913 19990219; JP 1999-311364 19991101.

AB A collagen-binding physiol. active polypeptide is provided. In this polypeptide, a peptide from fibronectin is ligated to a physiol. active peptide, and this hybrid polypeptide is provided with both the collagen-binding activity and the physiol. activity. A novel collagen matrix wherein the hybrid polypeptide is combined with collagen is also provided. The collagen-binding physiol. active polypeptide provided with both the collagen-binding activity and the physiol. activity is useful as a drug delivery system (DDS) of the physiol. active peptide. Furthermore, this polypeptide can be combined with collagen to provide a functionally modified collagen matrix which is quite useful as a new biomaterial adapted for use in tissue regeneration. Thus, chimeric proteins are constructed with an initiator methionine residue linked to the collagen-binding domain (residues 260-599) of human fibronectin, further linked to ligation residues Leu and Asp, followed by an ***enterokinase*** ***recognition*** /cleavage site (Asp-Asp-Asp-Lys), followed by the amino acid sequence for either human basic fibroblast growth factor or epidermal growth factor.

L14 ANSWER 14 OF 30 CAPLUS COPYRIGHT 2003 ACS

2000:368620 Document No. 133:13412 Protein expression vector with secretory signal sequence, Tag sequence, and cleavable sequence, and. Uemura, Hidetoshi; Okui, Akira; Kominami, Katsuya; Yamaguchi, Nozomi; Mitsui,

Shinichi (Fuso Pharmaceutical Industries, Ltd., Japan). PCT Int. Appl. WO 2000031284 A1 20000602, 44 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1999-JP6474 19991119. PRIORITY: JP 1998-331515 19981120.

AB A protein expression vector characterized by contg. a secretory signal sequence and, in the 3'-downstream side thereof, a Tag sequence, a scissile or cleavable sequence and a cloning site, into which a nucleic acid sequence encoding a target protein can be inserted, in this order, is disclosed. The vector may contain a spacer sequence 3' of the secretory signal sequence, which could be a scissile nucleic acid sequence. The scissile nucleic acid sequence is ***cleavable*** with ***enterokinase***, and the secretory signal sequence may be IgG(.kappa.) signal or a trypsin signal sequence. The Tag nucleic acid sequence is preferably a polyhistidine and may addnl. contain an epitope coding sequence. The target protein may be human active neurosin. Methods and compns. for producing a recombinant target protein, possibly as a ***fusion*** protein are also claimed. These expression vectors directs secretion of recombinant proteins into the culture medium of infected insect cells. By providing a vector-encoded signal peptide upstream from a multiple cloning site, the product of the inserted cDNA is directed to the secretory pathway. In addn., a C-terminal His-tag allows convenient purifn. of the native protein directly from the culture medium in less than 5 h. The His-tag can be cleaved off the purified protein by utilizing an enterokinase cleavage site located directly C-terminal to the His sequence. By insertion of a coding sequence representing the human active neurosin into the expression vectors, a high level of protein synthesis was demonstrated in COS-1 and Sf9 cells with either IgG(.kappa.) signal or a trypsin signal sequence. The high level of prodn. and the ease with which native protein can be purified almost to homogeneity, makes these expression vectors particularly suitable for protein synthesis and purifn.

L14 ANSWER 15 OF 30 CAPLUS COPYRIGHT 2003 ACS

2000:273190 Document No. 133:295074 Construction and characterization of a novel recombinant single-chain variable fragment antibody against Western equine encephalitis virus. Long, Melissa C.; Jager, Scott; Mah, Dave C. W.; Jebailey, Lellean; Mah, Maria A.; Masri, Saad A.; Nagata, Les P. (Medical Countermeasures Section, Defence Research Establishment Suffield, Medicine Hat, AB, T1A 8K6, Can.). Hybridoma, 19(1), 1-13 (English) 2000. CODEN: HYBRDY. ISSN: 0272-457X. Publisher: Mary Ann Liebert, Inc..

AB A novel recombinant single-chain fragment variable (scFv) antibody against Western equine encephalitis virus (WEE) was constructed and characterized. Using antibody phage display technol., a scFv was generated from the WEE specific hybridoma, 10B5 E7E2. The scFv was fused to a human heavy chain IgG1 const. region (CH1-CH3) and contained an intact 6 His tag and ***enterokinase*** ***recognition*** site (RS10B5huFc). The RS10B5huFc antibody was expressed in E. coli and purified by affinity chromatog. as a 70-kDa protein. The RS10B5huFc antibody was functional in binding to WEE antigen in indirect enzyme-linked immunosorbent assays (ELISAs). Furthermore, the RS10B5huFc antibody was purified in proper conformation and formed multimers. The addn. of the human heavy chain to the scFv replaced effector functions of the mouse antibody. The Fc domain was capable of binding to protein G and human complement. The above properties of the RS10B5huFc antibody make it an excellent candidate for immunodetection and immunotherapy studies.

L14 ANSWER 16 OF 30 CAPLUS COPYRIGHT 2003 ACS

1999:461847 Document No. 131:224110 S. pombe expression vector with 6.times.(His) tag for protein purification and potential for ligation-independent cloning. Hosfield, Tanya; Lu, Quinn (Stratagene Cloning Systems, La Jolla, CA, USA). BioTechniques, 27(1), 58-60 (English) 1999. CODEN: BTNQDO. ISSN: 0736-6205. Publisher: Eaton Publishing Co..

AB A new plasmid vector, pESP-2, was constructed and tested for gene expression and protein prodn. in Schizosaccharomyces pombe. This vector

contains the S. pombe inducible nmt1 promoter for high-level gene expression and the 6.times.(His) affinity tag for protein purifn. The vector contains a multiple cloning site with unique restriction enzyme sites and ligation-independent cloning (LIC) sites. The LIC sites enable direction cloning of a PCR product immediately downstream of an ***enterokinase*** ***recognition*** site. Thus, polypeptides without extraneously added amino acids can be obtained by removing the purifn. tag with enterokinase after purifn. of the recombinant 6.times.(His) ***fusion*** protein.

L14 ANSWER 17 OF 30 CAPLUS COPYRIGHT 2003 ACS

1999:322239 Document No. 131:165983 An Escherichia coli Expression Vector That Allows Recovery of Proteins with Native N-Termini from Purified Calmodulin-Binding Peptide ***Fusions***. Wyborski, Denise L.; Bauer, John C.; Zheng, Chao-Feng; Felts, Katherine; Vaillancourt, Peter (Stratagene Cloning Systems, La Jolla, CA, 92037, USA). Protein Expression and Purification, 16(1), 1-10 (English) 1999. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Academic Press.

AB We describe a T7-based Escherichia coli expression vector in which protein coding sequence is seamlessly fused to the N-terminal calmodulin-binding peptide (CBP) purifn. tag. We combined the use of the site-specific protease ***enterokinase*** (EK) and the type IIs restriction enzyme Eam1104 I, which ***cleave*** outside their resp. (amino acid and nucleotide) target sequences, such that any amino acid sequence may be fused directly C-terminal to the EK cleavage site without codon constraints conferred by the cloning method. PCR products are cloned using ligation-dependent or ligation-independent methods with high cloning efficiencies (>106 cfu/.mu.g vector), allowing prodn. of insert quantities sufficient for several cloning expts. with a limited no. of PCR cycles, resulting in a significant time-savings and reduced likelihood of accumulating PCR-derived mutations. CBP ***fusion*** proteins are expressed to high levels when the CBP peptide is positioned at the N-terminus. CBP binds to calmodulin with nanomolar affinity, and ***fusion*** proteins are purified to near homogeneity from crude exts. with one pass through calmodulin affinity resin using gentle binding and elution conditions. We show high efficiency seamless cloning of three inserts into the pCAL-n-EK vector, including one encoding the protein c-Jun N-terminal kinase (JNK). CBP-EK-JNK ***fusion*** protein was synthesized to 10-20 mg/L culture and purified to near homogeneity in one step with calmodulin affinity resin. The ***fusion*** tag was efficiently removed with EK to yield active JNK with native N-terminal amino acid sequence. (c) 1999 Academic Press.

L14 ANSWER 18 OF 30 CAPLUS COPYRIGHT 2003 ACS

1999:226539 Document No. 131:68807 A new series of pET-derived vectors for high efficiency expression of Pseudomonas exotoxin-based ***fusion*** proteins. Matthey, Barbel; Engert, Andreas; Klimka, Alexander; Diehl, Volker; Barth, Stefan (Laboratory of Immunotherapy, Dep. I of Internal Medicine, University Hospital of Cologne, Cologne, 50931, Germany). Gene, 229(1-2), 145-153 (English) 1999. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier Science B.V..

AB Recombinant immunotoxins (rITs) are highly specific anti-tumor agents composed of monoclonal antibody fragments or other specific carriers coupled to plant or bacterial toxins. A major problem in the purifn. of rITs is the low periplasmic yield in currently available expression systems. Thus, the aim of this study was the development of a new bacterial expression system for high-level prodn. of rITs. We constructed a series of pET-based vectors for pelB-directed periplasmic secretion or cytoplasmic prodn. under the control of the T7lac promoter. Expression in Escherichia coli BL21 (DE3)pLysS allowed a tightly regulated iso-Pr .beta.-d-thiogalactopyranoside (IPTG) induction of protein synthesis. An ***enterokinase*** - ***cleavable*** poly-histidine cluster was introduced into this setup for purifn. by affinity chromatog. A major modification resulted from the insertion of a specifically designed multiple cloning site. It contains only rare restriction enzyme recognition sites used for cloning of Ig variable region genes, as well as unique SfiI and NotI restriction sites for directed insertion of single-chain variable fragments (scFv) available from established bacteriophage systems. For this purpose, we deleted two naturally occurring internal SfiI consensus sites in a deletion mutant of Pseudomonas aeruginosa exotoxin A (ETA'). Each single structural element

of the new vector (promoter, leader sequence, purifn. tag, scFv sequence, selectable marker, and toxin gene) was flanked by unique restriction sites allowing simple directional substitution. The fidelity of IPTG induction and high-level expression were demonstrated using an anti-CD30 scFv (Ki-4) fused to ETA'. These data confirm a bacterial vector system esp. designed for efficient periplasmic expression of ETA'-based ***fusion*** toxins.

L14 ANSWER 19 OF 30 CAPLUS COPYRIGHT 2003 ACS

1998:425478 Document No. 129:172722 Production of biologically active salmon calcitonin in the milk of transgenic rabbits. McKee, Colin; Gibson, Allan; Dalrymple, Mike; Emslie, Liz; Garner, Ian; Cottingham, Ian (PPL Therapeutics Ltd., Edinburgh, EH25 9PP, UK). Nature Biotechnology, 16(7), 647-651 (English) 1998. CODEN: NABIF9. ISSN: 1087-0156. Publisher: Nature America.

AB Salmon calcitonin (sCT) is an example of one of the many bioactive peptides that require amidation of the carboxy terminus for full potency. We describe a method for the prodn. of amidated sCT in the mammary gland of transgenic rabbits. Expression of a ***fusion*** protein comprising human alpha lactalbumin joined by an ***enterokinase*** ***cleavable*** linker to sCT was directed to the mammary gland under the control of the ovine beta lactoglobulin promoter. C-terminal amidation in vivo was achieved by extending the sCT by a single glycine residue that provides a substrate for endogenous amidating activity in the mammary gland. Full characterization of the released sCT demonstrated it to be equiv. to synthetic std. in terms of structure, purity, and potency.

L14 ANSWER 20 OF 30 CAPLUS COPYRIGHT 2003 ACS

1998:296766 Document No. 129:64697 A thioredoxin ***fusion*** protein of VanH, a D-lactate dehydrogenase from Enterococcus faecium: cloning, expression, purification, kinetic analysis, and crystallization. Stoll, Vincent S.; Manohar, A. Vaito; Gillon, Wanda; Macfarlane, Emma L. A.; Hynes, Rosemary C.; Pai, Emil F. (Department of Biochemistry, University of Toronto, Toronto, ON, M5S 1A8, Can.). Protein Science, 7(5), 1147-1155 (English) 1998. CODEN: PRCIEI. ISSN: 0961-8368. Publisher: Cambridge University Press.

AB The gene encoding the vancomycin resistance protein VanH from Enterococcus faecium, a D-lactate dehydrogenase, has been cloned into a thioredoxin expression system (pTRxFus) and expressed as a ***fusion*** protein. The use of several other expression systems yielded only inclusion bodies from which no functional protein could be recovered. Expts. to remove the thioredoxin moiety by ***enterokinase*** cleavage at the engineered ***recognition*** site under a variety of conditions resulted in nonspecific proteolysis and inactivation of the protein. The intact ***fusion*** protein was, therefore, used for kinetic studies and crystn. trials. It has been purified to greater than 90% homogeneity by ammonium sulfate pptn. followed by Ph Sepharose chromatog. Based on kcat/KM for pyruvate, it is 20% as active as native VanH. Michaelis consts. for NADPH, NADH, and pyruvate, of .apprx.3.5 .mu.M, 19.0 .mu.M, and 1.5 mM, resp., were comparable to those reported for the native VanH (Bugg TDH et al., 1991, Biochem. 30:10408-10415). Like native VanH, max. activity of the ***fusion*** protein requires the presence of an anion (phosphate or acetate); however, in addn., a strongly reducing environment is needed for optimal efficacy. Competitive inhibition consts. for ADP-ribose, NAD+, and oxamate have also been detd. Crystn. by hanging drop vapor diffusion produced two different crystal forms, one hexagonal and the other tetragonal. Flash-frozen crystals of the tetragonal form diffracted to 3.0 .ANG. resoln. at a synchrotron radiation source.

L14 ANSWER 21 OF 30 CAPLUS COPYRIGHT 2003 ACS

1997:707262 Document No. 128:10990 Influence of a NH2-terminal extension on the activity of KTX2, a K+ channel blocker purified from Androctonus australis scorpion venom. Legros, Christian; Feyfant, Eric; Sampieri, Francois; Rochat, Herve; Bougis, Pierre E.; Martin-Eauclaire, Marie-France (Laboratoire de Biochimie, Ingenierie des Proteines, UMR 6560 du Centre National de la Recherche Scientifique, Institut Federatif Jean Roche, Faculte de Medecine Nord, Boulevard Pierre Dramard, Marseille, 13916/20, Fr.). FEBS Letters, 417(1), 123-129 (English) 1997. CODEN: FEBLAL. ISSN: 0014-5793. Publisher: Elsevier.

AB A cDNA encoding a short polypeptide blocker of K+ channels, kaliotoxin 2 (KTX2), from the venom of the North African scorpion Androctonus australis

was expressed in the periplasmic space of *Escherichia coli*. KTX2 was produced as a ***fusion*** protein with the maltose binding protein followed by the ***recognition*** site for factor Xa or ***enterokinase*** preceding the first amino acid residue of the toxin. The fully refolded recombinant KTX2 (rKTX2) was obtained (0.15-0.30 mg/L of culture) and was indistinguishable from the native toxin according to chem. and biol. criteria. An N-extended analog of KTX2 exhibiting three addnl. residues was also expressed. This analog had 1000-fold less affinity for the 125I-kaliotoxin binding site on rat brain synaptosomes than KTX2. Conformational models of KTX2 and its mutant were designed by amino acid replacement using the structure of agitoxin 2 from *Leiurus quinquestriatus* as template, to try to understand the decrease in affinity for the receptor.

L14 ANSWER 22 OF 30 CAPLUS COPYRIGHT 2003 ACS

1996:696040 Document No. 126:30362 Production of recombinant growth hormone releasing factor via post-translational C-terminal .alpha.-amidation. Henriksen, D. B.; Stout, J. S.; Partridge, B. E.; Holmquist, B.; Wagner, F. W. (BioNebraska, Inc., Lincoln, NE, 68524, USA). Peptides: Chemistry, Structure and Biology, Proceedings of the American Peptide Symposium, 14th, Columbus, Ohio, June 18-23, 1995, Meeting Date 1995, 651-652. Editor(s): Kaumaya, Pravin T. P.; Hodges, Robert S. Mayflower Scientific: Kingswinford, UK. (English) 1996. CODEN: 63NTAF.

AB A process involving 3 enzymic steps and a 2-stage HPLC purifn. has been used to prep. multi-gram amts. of injectable grade GRF(1-44)NH2. GRF(1-44) contg. a C-terminal Ala extension has been expressed in *Escherichia coli* as a ***fusion*** protein linked to human carbonic anhydrase (HCA) through an interlinking peptide contg. ***recognition*** sites for thrombin and ***enterokinase***. The Ala extension provides an enzymic recognition site for post-translational .alpha.-amidation. Incorporation of the peptide onto HCA affords protection against proteolytic degradn.

L14 ANSWER 23 OF 30 CAPLUS COPYRIGHT 2003 ACS

1995:934612 Document No. 124:46816 Versatile, multi-featured plasmids for high-level expression of heterologous genes in *Escherichia coli*: overproduction of human and murine cytokines. Mertens, Nico; Remaut, Erik; Fiers, Walter (Laboratory of Molecular Biology, University of Gent, B-9000, Ghent, Belg.). Gene, 164(1), 9-15 (English) 1995. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier.

AB We describe the construction, expression characteristics and some applications of a versatile dual-promoter expression plasmid for heterologous gene expression in *Escherichia coli* which contains both .lambda. pL and PT7 promoters. Furthermore, the plasmid is optimized to allow the expression of mature coding sequences without compromising the strength of the highly efficient PT7 or of the T7g10 ribosome-binding site. The effect of the naturally occurring RNA loops at both the 5' and 3' ends of the T7g10 mRNA on expression was also examd. A double T7 RNA polymerase transcription terminator was inserted to ensure more reliable transcription termination and a higher expression level of the preceding gene. Further improvements involve a clockwise orientation of the promoters to minimize read-through transcription from plasmid promoters, a largely extended multiple cloning site, an antisense phage T3 promoter and a phage fl-derived, single-stranded replication origin. Variants of this plasmid allow for the prodn. of ***fusion*** proteins with part of T7g10, a hexahistidine peptide and an ***enterokinase*** ***recognition*** site. The potential of these expression vectors is demonstrated by comparing the expression levels of a no. of mammalian cytokines (human tumor necrosis factor, human immune interferon, human and murine interleukins 2, murine interleukin 4 and murine fibroblast interferon), using these expression plasmids.

L14 ANSWER 24 OF 30 CAPLUS COPYRIGHT 2003 ACS

1995:774399 Document No. 123:167663 Production of recombinant bovine enterokinase catalytic subunit in *Escherichia coli* using the novel secretory ***fusion*** partner DsbA. Collins-Rcie, Lisa A.; McColgan, James M.; Grant, Kathleen L.; DiBlasio-Smith, A.; McCoy, John M.; LaVallie, Edward R. (Genetics Institute, Cambridge, MA, 02140, USA). Bio/Technology, 13(9), 982-7 (English) 1995. CODEN: BTCHDA. ISSN: 0733-222X. Publisher: Nature Publishing Co..

AB Enterokinase (EK) is a heterodimeric serine protease which plays a key

role in initiating the proteolytic digestion cascade in the mammalian duodenum. The enzyme acts by converting trypsinogen to trypsin via a highly specific cleavage following the pentapeptide recognition sequence (Asp)⁴-Lys. This stringent site specificity gives EK great potential as a ***fusion*** protein cleavage reagent. Recently, a cDNA encoding the catalytic (light) chain of bovine enterokinase (EKL), was identified, characterized, and transiently expressed in mammalian COS cells. The authors report here the prodn. of EKL in Escherichia coli by a novel secretory expression system that utilizes E. coli DsbA protein as an N-terminal ***fusion*** partner. The EKL cDNA was fused in-frame to the 3'-end of the coding sequence for DsbA, with the two domains of the ***fusion*** protein sepd. by a linker sequence encoding an ***enterokinase*** ***recognition*** site. Active, processed recombinant EKL (rEKL) was generated from this ***fusion*** protein via an autocatalytic cleavage reaction. The enzymic properties of the bacterially produced rEKL were indistinguishable from the previously described COS-derived enzyme. Both forms of rEKL were capable of cleaving peptides, polypeptides and trypsinogen with the same specificity exhibited by the native heterodimeric enzyme purified from bovine duodena. Interestingly, rEKL activated trypsinogen poorly relative to the native heterodimeric enzyme, but was superior in its ability to cleave artificial ***fusion*** proteins contg. the (Asp)⁴-Lys recognition sequence.

L14 ANSWER 25 OF 30 CAPLUS COPYRIGHT 2003 ACS

1994:427987 Document No. 121:27987 Introduction of arbitrary sequences into genes by use of class IIs restriction enzymes. Beck, Reinhard; Burtscher, Helmut (Boehringer Mannheim Res. Cent., Penzberg, D-82372, Germany). Nucleic Acids Research, 22(5), 886-7 (English) 1994. CODEN: NARHAD. ISSN: 0305-1048.

AB The authors wanted to insert a DNA sequence coding for four histidine residues and an enterokinase cleavage site between the signal sequence and the mature sequence of human placental alk. phosphatase for purifn. The authors added the ***enterokinase*** ***recognition*** site in order to allow regeneration of the authentic N-terminus of the enzyme. Here the authors describe a convenient way to achieve this, using class IIs restriction endonucleases. Class IIs restriction endonucleases cut DNA several nucleotides away from their recognition site irresp. of the intervening sequence. This can be exploited to generate arbitrary sticky ends for in-frame ***fusion*** of DNA sequences, combining two PCR reactions with a simple cloning step. The authors designed two oligonucleotide primers (ON2, ON3) contg. the desired sequences, a SapI restriction site and some addnl. nucleotides (including extra restriction endonuclease cleavage sites for other purposes). The whole procedure takes about 2 to 3 days from PCR to transformation of cells. This method works with fragments of a wide range of sizes, fragments can be inserted, deleted or fused regardless of their individual size differences. The protocol is very simple, using std. PCR conditions for introduction of a class IIs restriction enzyme recognition site and easy ligation of the sticky ends created by cleavage at the "transient" site.

L14 ANSWER 26 OF 30 CAPLUS COPYRIGHT 2003 ACS

1994:268195 Document No. 120:268195 Combinatorial polypeptide antigens. Crea, Roberto (Creagen, Inc., USA). PCT Int. Appl. WO 9400151 A1 19940106, 49 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1993-US5899 19930618. PRIORITY: US 1992-900123 19920618.

AB A set of polypeptide antigens having amino acid sequences derived from amino acid sequences of a population of variants of a protein, or a portion thereof, is produced by (1) selecting a protein, or a portion thereof, which exhibits a population of N variants, represented by the formula A₁A₂A₃ ... A_{n-2}A_{n-1}A_n, where A_n is an amino acid occurring at amino acid position n of the protein, or portion thereof; (2) detg. the no. of times Onaa each type of amino acid occurs at each amino acid position n in the N variants; (3) calcg. the frequency of occurrence (Onaa/N)_n of each type of amino acid at each amino acid position n in the N variants; and (4) generating a set of polypeptide antigens having amino acid sequences represented substantially by the formula A'₁A'₂A'₃ ... A'_{n-2}A'_{n-1}A'_n, where A'_n is defined as an amino acid type which occurs at greater than a selected frequency at the corresponding amino acid position in the N variants. Thus, a vaccine directed to envelope glycoprotein

gp120 of HIV-1, which shows marked diversity between isolates, was produced by tabulating the frequency of occurrence of amino acids at each of the positions in the V3 loop (residues 303-338), deriving a degenerate oligonucleotide sequence encoding .gtoreq.90% of the variants, synthesizing this oligonucleotide by ligating 6 shorter oligonucleotides, amplifying by PCR to a V3 loop gene library, fusing in-frame with an ***enterokinase*** cleavage ***recognition*** sequence, cloning and expression in Escherichia coli, and removal of protein A sequences from the ***fusion*** protein with enterokinase.

L14 ANSWER 27 OF 30 CAPLUS COPYRIGHT 2003 ACS

1988:623979 Document No. 109:223979 Cloning and expression of cDNA for human apolipoprotein or variant in Escherichia coli. Lorenzetti, Rolando; Monaco, Lucia; Soria, Marco; Palomba, Raffaele; Isacchi, Antonella; Sarmientos, Paolo (Farmitalia Carlo Erba S.p.A., Italy). Eur. Pat. Appl. EP 267703 A1 19880518, 30 pp. DESIGNATED STATES: R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1987-309318 19871021. PRIORITY: GB 1986-25435 19861023.

AB The cDNA for human apolipoprotein A1 (apoA1) and its variants are cloned and expressed in Escherichia coli. Plasmid pLM8 was constructed contg. the protein A gene fused to the mature human apoA1 cDNA. The binding affinity of the recombinant protein to J774 or Fao cell surface receptors was 3.5-4.9 .times. 10⁻⁸ M, compared with 2.8-3.0 .times. 10⁻⁸ M for high-d. lipoprotein purified from human plasma.

L14 ANSWER 28 OF 30 CAPLUS COPYRIGHT 2003 ACS

1988:525454 Document No. 109:125454 Immunoaffinity purification system. Hopp, Thomas P.; Prickett, Kathryn S. (Immunex Corp., USA). PCT Int. Appl. WO 8804692 A1 19880630, 17 pp. DESIGNATED STATES: W: AU, DK, JP, KR; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1987-US3113 19871204. PRIORITY: US 1986-944261 19861219.

AB An immunoaffinity protein purifn. system comprises a divalent cation-dependent monoclonal antibody and a recombinant ***fusion*** protein contg. the protein of interest fused to a polyanionic peptide which the antibody recognizes. Monoclonal antibody from hybridoma 4E11 was prepd. and attached to Affigel-10. Culture broth of yeast transformed with pBC65, which encodes a DYKDDDDK-.alpha. mating factor leader peptide-burst-forming factor ***fusion*** protein, was added to the prepd. immunoaffinity gel. The ***fusion*** protein-contg. broth, immunoaffinity gel, and washing buffer contained 0.5 mM MgCl₂ and 1.0 mM CaCl₂. The protein was eluted with 0.1M glycine HCl.

L14 ANSWER 29 OF 30 CAPLUS COPYRIGHT 2003 ACS

1987:208794 Document No. 106:208794 Plasmid vector containing a signal for specific cleavage of chimeric proteins. Preparation of [Leu5] enkephalin with the aid of enteropeptidase. Dobrynin, V. N.; Boldyreva, E. F.; Filippov, S. A.; Chuvpilo, S. A.; Korobko, V. G.; Vorotyntseva, T. I.; Bessmertnaya, L. Ya.; Mikhailova, A. G.; Amerik, A. Yu.; Antonov, V. K. (M. M. Shemyakin Inst. Bioorg. Chem., Moscow, USSR). Bioorganicheskaya Khimiya, 13(1), 119-21 (Russian) 1987. CODEN: BIKHD7. ISSN: 0132-3423.

AB Plasmid vector pEK1 was constructed such that it contains a enteropeptidase (EC 3.4.21.9) [***9014-74-8***] ***recognition*** site-encoding region within a .beta.-galactosidase [9031-11-2] gene. Synthetic DNA encoding [Leu5]enkephalin [58822-25-6] was fused to the .beta.-galactosidase gene at this site after restriction by KpnI, and the recombinant plasmid pEK-ENK was used to transform Escherichia coli. Transformant clones formed the fused protein at 30% of total protein yield when induced with isopropylthio-.beta.-D-galactopyranoside. After ion-exchange fractionation the chimeric protein was cleaved with enteropeptidase to yield [Leu5]enkephalin.

L14 ANSWER 30 OF 30 CAPLUS COPYRIGHT 2003 ACS

1987:28521 Document No. 106:28521 Improved expression using fused genes providing for protein product. Cousens, Lawrence S.; Tekamp-Olson, Patricia A.; Shuster, Jeffrey R.; Merryweather, James P. (Chiron Corp., USA). Eur. Pat. Appl. EP 196056 A2 19861001, 36 pp. DESIGNATED STATES: R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1986-104066 19860325. PRIORITY: US 1985-717209 19850328.

AB A method for enhancing the prodn. of heterologous proteins in fungi by

recombinant DNA techniques involves ***fusion*** of a gene encoding a heterologous protein produced in large amt. and in stable form in the host to a sequence encoding a desired heterologous protein, where the hybrid proteins produced are joined by a selectively cleavable linkage. Plasmid pYAS11 was constructed which contains the human superoxide dismutase gene fused to the amino terminus of the human proinsulin gene, with a methionine codon at the junction, under the control of the hybrid inducible ADH2-GAP promoter and the GAP terminator. The ***fusion*** protein produced by yeast transformants accounts for .gtoreq.10% of the total cell protein. After cleavage of the hybrid protein at the methionine junction using CNBr and formic acid in water, the proinsulin was converted to its S-sulfonate form in the presence of urea, Na sulfite, and Na tetrathionate, and was purified on an ion-exchange column. Proinsulin-S-sulfonate obtained was 90% pure, and the yield was 150 mg protein/124 g yeast.

=> S L13 NOT L14
L15 6 L13 NOT L14

=> D 1-6 CBIB ABS

L15 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2003 ACS

2002:228059 Document No. 137:17104 Engineered Recombinant Enteropeptidase Catalytic Subunit: Effect of N-Terminal Modification. Song, Hye-Won; Choi, Sung-Il; Seong, Baik L. (Yonsei Engineering Research Center B120E, Protheon Incorporated, Yonsei University, Seoul, 120-749, S. Korea). Archives of Biochemistry and Biophysics, 400(1), 1-6 (English) 2002. CODEN: ABBIA4. ISSN: 0003-9861. Publisher: Elsevier Science.

AB Enteropeptidase (***enterokinase***) is a serine protease highly specific for ***recognition*** and cleavage of the target sequence of Asp-Asp-Asp-Asp-Lys (D4K). The three-dimensional structure of the enteropeptidase shows that the N-terminal amino acid is buried inside the protein providing mol. interactions necessary to maintain the conformation of the active site. To det. the influence of the N-terminal amino acid of enteropeptidase light chain (EKL) on the enzymic activity, we constructed various mutants including 17 different single amino acid substitutions and three different extensions at the N-terminal end. The mutants of recombinant enteropeptidase (rEKL) were expressed in Saccharomyces cerevisiae and secreted into culture medium. Among 20 different mutants tested, only the mutant with the Ile .fwdarw. Val substitution exhibited significant activity. The kinetic properties of the mutant protein were very similar to those of the wild-type rEKL. Based on the three-dimensional structure where the N-terminal Ile is oriented into hydrophobic pocket, the results suggest that Val could substitute for Ile without affecting the active conformation of the enzyme. The results also explain why all trypsin-like serine proteases exclusively carry either Ile or Val at the N-termini. Moreover, this finding provides a framework for expressing the N-terminally engineered enteropeptidase in Escherichia coli, utilizing the known property of the methionine aminopeptidase that exhibits poor activity toward the N-terminal Met-Ile bond, but offers efficient cleavage of the Met-Val bond.

L15 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2003 ACS

1998:106052 Document No. 128:163644 A homogeneous nucleic acid detection method utilizing simultaneous target and signal amplification. Hepp, Jozsef; Lengyel, Zsolt; Pande, Rajiv; Botyanszki, Janos; Sahin-Toth, Miklos (Navix, Inc., USA; Hepp, Jozsef; Lengyel, Zsolt; Pande, Rajiv; Botyanszki, Janos; Sahin-Toth, Miklos). PCT Int. Appl. WO 9804739 A2 19980205, 73 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US12415 19970716. PRIORITY: US 1996-692825 19960725.

AB A method for detg. the presence of a target nucleic acid in a sample using a two-stage target cycling reaction is described. The method uses a hybridization probe that is complexed with an activator. When the probe hybridizes with its target the activator is released. The activator then

interacts with an analog of the target sequence that is immobilized via an anchor moiety, leading to its release and the generation of a signal specific to the released cleavage products. The released target analog then restarts the cyclic reaction by binding to a second probe, which effectuates release of a second activator, and so on. This cyclic reaction amplifies the signal generated from a single target nucleic acid mol. in the sample, which greatly enhances the level of target detection that can be expected. The analog and the target have to be sepd. in the assay and this can be brought about by immobilizing them on sep. surfaces or using a membrane that is permeable to the released activator and target analog, but not the target or the free probe. An enzyme-based version of the assay is demonstrated. An oligonucleotide probe with a central oligoribonucleotide was prepd., conjugated with enterokinase, and immobilized. The anchored target analog was conjugated to trypsinogen. When the target sequence hybridizes to the enterokinase labeled probe, it is cleaved at the RNA moiety with RNase H to release the enterokinase. The released ***enterokinase*** ***cleaves*** the trypsinogen to release the oligonucleotide. The resulting trypsin can be assayed with a chromogenic substrate.

L15 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2003 ACS

1994:599404 Document No. 121:199404 Enterokinase, the initiator of intestinal digestion, is a mosaic protease composed of a distinctive assortment of domains. Kitamoto, Yasunori; Yuan, Xin; Wu, Qingyu; McCourt, David W.; Sadler, J. Evan (Dep. Med. Biochem. Molecular Biophysics, Washington Univ. Sch. Med., St. Louis, MO, 63110, USA). Proceedings of the National Academy of Sciences of the United States of America, 91(16), 7588-92 (English) 1994. CODEN: PNASA6. ISSN: 0027-8424.

AB ***Enterokinase*** is a protease of the intestinal brush border that specifically ***cleaves*** the acidic propeptide from trypsinogen to yield active trypsin. This cleavage initiates a cascade of proteolytic reactions leading to the activation of many pancreatic zymogens. The full-length cDNA sequence for bovine enterokinase and partial cDNA sequence for human enterokinase were detd. The deduced amino acid sequences indicate that active two-chain enterokinase is derived from a single-chain precursor. Membrane assocn. may be mediated by a potential signal-anchor sequence near the amino terminus. The amino terminus of bovine enterokinase also meets the known sequence requirements for protein N-myristoylation. The amino-terminal heavy chain contains domains that are homologous to segments of the low d. lipoprotein receptor, complement components C1r and C1s, the macrophage scavenger receptor, and a recently described motif shared by the metalloprotease meprin and the Xenopus A5 neuronal recognition protein. The carboxyl-terminal light chain is homologous to the trypsin-like serine proteases. Thus, enterokinase is a mosaic protein with a complex evolutionary history. The amino acid sequence surrounding the amino terminus of the enterokinase light chain is ITPK-IVGG (human) or VSPK-IVGG (bovine), suggesting that single-chain ***enterokinase*** is activated by an unidentified trypsin-like protease that ***cleaves*** the indicated Lys-Ile bond. Therefore, enterokinase may not be the "first" enzyme of the intestinal digestive hydrolase cascade. The specificity of enterokinase for the DDDDK-I sequence of trypsinogen may be explained by complementary basic-amino acid residues clustered in potential S2-S5 subsites.

L15 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2003 ACS

1993:487512 Document No. 119:87512 Interaction cloning: identification of a helix-loop-helix zipper protein that interacts with c-Fos. Blanar, Michael A.; Rutter, William J. (Hormone Res. Inst., Univ. California, San Francisco, CA, 94143, USA). Science (Washington, DC, United States), 256(5059), 1014-18 (English) 1992. CODEN: SCIEAS. ISSN: 0036-8075.

AB A facile method for isolating genes that encode interacting proteins has been developed with a polypeptide probe that contains an amino-terminal extension with recognition sites for a monoclonal antibody, a specific endopeptidase, and a site-specific protein kinase. This probe, contg. the basic region-leucine zipper dimerization motif of c-Fos, was used to screen a cDNA library. A cDNA that encoded a member of the basic-helix-loop-helix-zipper (bHLH-Zip) family of proteins was isolated. The cDNA-encoded polypeptide FIP (Fos interacting protein) bound to oligonucleotide probes that contained DNA binding motifs for other HLH proteins. When cotransfected with c-Fos, FIP stimulated transcription of an AP-1-responsive promoter.

L15 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2003 ACS

1992:548045 Document No. 117:148045 The cloned platelet thrombin receptor couples to at least two distinct effectors to stimulate phosphoinositide hydrolysis and inhibit adenylyl cyclase. Hung, David T.; Wong, Yung H.; Vu, Thien Khai H.; Coughlin, Shaun R. (Dep. Lab. Med., Univ. California, San Francisco, CA, 94143, USA). Journal of Biological Chemistry, 267(29), 20831-4 (English) 1992. CODEN: JBCHA3. ISSN: 0021-9258.

AB Thrombin both stimulates phosphoinositide hydrolysis and inhibits adenylyl cyclase in a variety of cell types. Whether the cloned human platelet thrombin receptor accounts for both of these signaling events is unknown. The thrombin receptor agonist peptide causes both phosphoinositide hydrolysis and inhibition of adenylyl cyclase in naturally thrombin-responsive CCL-39 cells. To exclude the possibility that the agonist peptide or thrombin itself may activate these pathways via distinct receptors and to circumvent a lack of suitable thrombin receptor-null cells, the authors utilized a designed ***enterokinase*** receptor, a thrombin receptor with its thrombin cleavage ***recognition*** sequence LDPR replaced by DDDDK, the ***enterokinase*** cleavage ***recognition*** sequence. Transfection of enterokinase-unresponsive cells with this construct conferred both enterokinase-sensitive phosphoinositide hydrolysis and inhibition of adenylyl cyclase. The phosphoinositide hydrolysis response was largely insensitive to pertussis toxin, whereas the adenylyl cyclase response was completely blocked by pertussis toxin. Thus, the cloned thrombin receptor can effect both phosphoinositide hydrolysis and inhibition of adenylyl cyclase via .gtoreq.2 distinct effectors, most likely Gq-like and Gi-like G-proteins.

L15 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2003 ACS

1982:156035 Document No. 96:156035 Additional observations on cholecystokinin and the vasoactive intestinal polypeptide. Mutt, Viktor (Dep. Biochem. II, Karolinska Inst., Stockholm, S-104 01, Swed.). Peptides (New York, NY, United States), Volume Date 1981, 2(Suppl. 2, Brain-Gut Axis: New Front.), 209-14 (English) 1982. CODEN: PPTDD5. ISSN: 0196-9781.

AB An attempt was made to reactivate oxidized cholecystokinin (CCK) [9011-97-6] by redn. at room temp. with N-methylmercaptoacetamide [20938-74-3]. The reducing agent of choice for the redn. of methionine sulfoxide to methionine, has not been unequivocally successful, but the results seem promising. In the case of oxidized VIP [37221-79-7] and of oxidized tetragastrin [1947-37-1], redn. with N-methylmercaptoacetamide seems to result in reconversion of the peptides to their preoxidn. states, as evidenced by TLC. ***enterokinase*** [***9014-74-8***] ***Cleaves*** 39-CCK and 33-CCK with release of 8-CCK and the tetrapeptide immediately preceding it in the peptide chain. The conversion of 39-CCK to 33-CCK by the action of dipeptidyl aminopeptidase I [9032-68-2] has been confirmed.

=> S AVIDIN;S ANTIBODY;S KT3;S MYC;S FLAG PROTEIN

6679 AVIDIN
3489 AVIDINS

L16 7805 AVIDIN
(AVIDIN OR AVIDINS)

247286 ANTIBODY
272554 ANTIBODIES
375709 ANTIBODY

L17 (ANTIBODY OR ANTIBODIES)

L18 109 KT3

13376 MYC
10 MYCS

L19 13378 MYC
(MYC OR MYCS)

2768 FLAG
184 FLAGS
2911 FLAG

(FLAG OR FLAGS)

1514903 PROTEIN

1028922 PROTEINS

1751755 PROTEIN

(PROTEIN OR PROTEINS)

L20 18 FLAG PROTEIN

(FLAG(W) PROTEIN)

=> S L6 AND (L16,L17,L18,L19,L20)

L21 86 L6 AND ((L16 OR L17 OR L18 OR L19 OR L20))

=> S L6(10A) (L16,L17,L18,L19,L20)

L22 14 L6(10A) ((L16 OR L17 OR L18 OR L19 OR L20))

=> S L22 NOT L13

L23 12 L22 NOT L13

=> D 1-12 CBIB ABS

L23 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2003 ACS

2002:658665 Document No. 137:200272 Human prostate-assocd. protease HUPAP, polynucleotides, and antibodies for diagnosis, prognosis and treatment of prostatic and gastrointestinal disorders. Bandman, Olga; Lal, Preeti G. (USA). U.S. Pat. Appl. Publ. US 2002119531 A1 20020829, 27 pp., Cont.-in-part of U.S. 6,350,448. (English). CODEN: USXXCO. APPLICATION: US 2001-988975 20011119. PRIORITY: US 1997-807151 19970227; US 2000-478957 20000107.

AB The present invention provides a human prostate-assocd. protease or kallikrein (HUPAP), polynucleotides which encode HUPAP and antibodies which specifically bind HUPAP. The invention also provides expression vectors, host cells, agonists, antagonists, and antisense mols. The invention also provides methods for producing and using HUPAP and for treating disorders assocd. with expression of HUPAP, i.e. prostatic disorders such as prostate cancer and benign prostate hyperplasia, as well as gastrointestinal disorders such as cancers of esophagus, stomach, small intestine, large intestine and colon; congenital enterokinase deficiency; pancreatitis and ulcerative colitis.

L23 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2003 ACS

2000:688462 Document No. 133:265653 Protein isolation and analysis. Carr, Francis J. (Biovation Limited, UK). PCT Int. Appl. WO 2000057183 A1 20000928, 53 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-GB1015 20000317. PRIORITY: GB 1999-6551 19990323; GB 1999-7057 19990329; GB 1999-7641 19990406; GB 1999-14874 19990628; GB 1999-15363 19990702; GB 1999-15677 19990706; GB 1999-16511 19990714; GB 1999-20503 19990831; GB 1999-22285 19990921.

AB Novel methods for the identification and/or sequencing of proteins are provided. These methods are particularly suited to screening antibody libraries and in preferred embodiments make use of mass spectrometry techniques for direct or indirect sequencing.

L23 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2003 ACS

2000:608621 Document No. 133:191999 Method for regulating the stability of recombinant proteins, and antibodies and products useful therein. Chain, Daniel G. (Mindset Biopharmaceuticals (USA) Ltd., USA). PCT Int. Appl. WO 2000050089 A2 20000831, 69 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG,

CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US4749 20000225. PRIORITY: US 1999-PV122103 19990226.

- AB An antibody to a drug of interest is caused to be expressed in a target cell of interest by genetic therapy. This antibody is expressed along with a promoter and modulator for the antibody. The drug is administered to the patient, where it binds to the antibody for the drug until a crit. concn. of drug is reached at the target site. Once this crit. concn. of drug is achieved, the antibody is released from the drug/antibody conjugate, and the drug is available at the target site in concns. sufficient to treat the condition for which the drug is administered. In order to ensure that the antibodies are degraded at the proper time, the antibodies are designed to have built-in signals for degrdn.

L23 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2003 ACS

1994:649498 Document No. 121:249498 Cloning and expression of enterokinase-encoding sequences and manufacture of the protein for specific cleavage of fusion proteins. Lavallie, Edward R. (Genetics Institute, Inc., USA). PCT Int. Appl. WO 9416083 A1 19940721, 50 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US616 19940113. PRIORITY: US 1993-5944 19930115.

- AB Sequences encoding the catalytic domains of mammalian enterokinases are cloned and expressed for use in the specific cleavage of fusion proteins at the tetrapeptide (Asp)4Lys. The protein or the gene may also be used to treat enterokinase deficiencies (no data). Partial sequences for the bovine enzyme were obtained by PCR using amino acid sequence-derived oligonucleotides designed to minimize cross-hybridization with related sequences. Primers derived from these clones were used to amplify sequences from a bovine intestinal cDNA bank in .lambda.gt10 and the amplification product use as a probe. The sequence encoding the catalytic domain was obtained and expressed in CHO cells using the PACE gene signal sequence to direct secretion. Conditioned medium contained and enterokinase activity that was 100-fold less effective against trypsinogen than the native enzyme but 25-fold more effective against a fusion protein of Escherichia coli thioredoxin and human interleukin 11 than the full-length enterokinase.

L23 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2003 ACS

1992:123806 Document No. 116:123806 Monoclonal antibodies to human pancreatic trypsin 1 inhibit the activation of human trypsinogens 1 and 2. Guy-Crotte, Odette; Miszczuk-Jamska, Barbara; Brayle, Alex; Lafont, Pannie; Figarella, Catherine (Groupe Rech. Glandes Exocrines, Marseille, F-13009, Fr.). European Journal of Biochemistry, 204(1), 133-6 (English) 1992. CODEN: EJBCAI. ISSN: 0014-2956.

- AB Two monoclonal antibodies (Mabs) raised against human pancreatic trypsin 1 (Mab G6 and A8) were previously isolated and characterized. The 2 Mabs which recognize trypsinogen 1 were found to inhibit the activation of trypsinogen 1 by enterokinase. The inhibition of activation by the 2 Mabs was concn.-dependent, rapid, and virtually complete with Mab G6. The activation of trypsinogen 2 was totally inhibited by Mab G6, whereas Mab A8 had no effect on the activation of trypsinogen 2. The 2 Mabs had opposite effects on the proteolytic activity of trypsin 1; Mab G6 increased the proteolytic activity whereas Mab A8 inhibited trypsin activity by as much as 40%. This inhibition was concn.-dependent but could not account for the complete inhibition of activation of trypsinogen 1. Neither Mab significantly inhibited the esterolytic activity of either form of human trypsin. Western blot anal. of the reactivity of the 2 Mabs with trypsinogens of various species showed that only Mab G6 cross-reacted with dog trypsinogen.

L23 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2003 ACS

1990:629053 Document No. 113:229053 Monoclonal ***antibody*** against human ***enterokinase*** and immunohistochemical localization of the enzyme. Miyoshi, Yasuyuki; Onishi, Toshio; Sano, Toshiaki; Komi, Nobuhiko (Sch. Med., Univ. Tokushima, Tokushima, 770, Japan). Gastroenterologia Japonica, 25(3), 320-7 (English) 1990. CODEN: GAJABC. ISSN: 0435-1339.

- AB A monoclonal ***antibody***, hek-1, was raised against ***enterokinase*** or enteropeptidase that had previously been partially purified from human duodenal fluid. Hek-1 showed staining of two glycoprotein bands of relative mol. wts. of 260,000 and 240,000 on

immunoblot anal. of partially purified enterokinase and of the ammonium sulfate fraction of duodenal fluid. An enzyme immunoassay for human enterokinase was developed, making use of hek-1. Sensitivity to enterokinase was 20 times higher than that of the conventional assay where BAPA was used as a substrate. The immunohistochem. study with hek-1 showed staining of the brush border membrane and some goblet cells of the duodenum and upper jejunum but no staining of the colon epithelium.

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1989:529453 Document No. 111:129453 Preparation of a monoclonal

antibody against human ***enterokinase***. Miyoshi, Yasuyuki; Onishi, Toshio; Sano, Toshiaki; Komi, Nobuhiko (Sch. Med., Tokushima Univ., Tokushima, 770, Japan). Igaku no Ayumi, 149(13), 951-2 (Japanese) 1989. CODEN: IGAYAY. ISSN: 0039-2359.

AB A monoclonal ***antibody***, hek-1, against ***enterokinase*** (EK) purified partially from human duodenal fluid was prep'd. On immunoblotting analyses of the partially purified EK and ammonium sulfate fraction of duodenal fluid, hek-1 bound to 2 glycoproteins whose relative mol. wt. were 260,000 and 240,000 resp. The hek-1 showed staining of the brush border membrane and goblet cells in the human duodenum and upper jejunum, but no staining of the colon epithelium.

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1988:606177 Document No. 109:206177 The use of a 'universal' yeast expression vector to produce an antigenic protein of Mycobacterium leprae. Booth, Roger J.; Grandison, Prudence M.; Prestidge, Ross L.; Watson, James D. (Med. Sch., Univ. Auckland, Auckland, N. Z.). Immunology Letters, 19(1), 65-9 (English) 1988. CODEN: IMLED6. ISSN: 0165-2478.

AB This report describes the use of a recombinant yeast expression vector to synthesize and secrete the M. leprae 18 kDa antigenic protein. The protein is secreted with a short hydrophilic flag octapeptide fused to its amino-terminus. The fusion protein can be purified directly from yeast culture supernatant through an anti-flag ***antibody*** affinity column and the flag octapeptide removed using ***enterokinase***. The method provides a simple and rapid means of obtaining recombinant 18 kDa antigen in quantities suitable for immunol. studies.

L23 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2003 ACS

1986:494118 Document No. 105:94118 Immunoassays utilizing covalent conjugates of polymerized enzyme and antibody. Freytag, J. William; Ishikawa, Eiji (du Pont de Nemours, E. I., and Co., USA). Eur. Pat. Appl. EP 175560 A2 19860326, 24 pp. DESIGNATED STATES: R: BE, DE, FR, GB, IT, LU, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1985-306560 19850916. PRIORITY: US 1984-650868 19840914.

AB An enzyme is selectively crosslinked to form a polymd. enzyme which is covalently coupled to an antibody for use in immunometric assays. By using specific heterobifunctional or homobifunctional crosslinking agents, the conjugate can be produced in a highly reproducible fashion in a manner which provides for max. maintenance of enzymic and immunoreactivity and min. nonspecific adsorption. The resulting conjugate provides enhanced signal generation, shorter assay times, and higher overall sensitivity. Thus, .beta.-D-galactosidase was polymd. with o-phenylenedimaleimide and the maleimido-.beta.-galactosidase polymers conjugated to Fab'-SH fragments. The conjugate was used in an affinity column mediated immunometric assay for digoxin. It was shown that the larger the mol. wt. of the polymeric galactosidase, the greater the max. sensitivity that can be achieved. In addn., the greater the no. of antibody sites bound/polymer, the lower the background blank activity. The use of prepolymd. Fab'-.beta.-galactosidase conjugate (enzyme mol. wt. = 20,000; Fab'/polymer enzyme molar ratio of 20) resulted in a 20-30-fold increase in sensitivity vs. a monomer conjugates.

L23 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2003 ACS

1986:475419 Document No. 105:75419 Polymeric enzyme-antibody complexes for enzyme immunoassay. Jei, Uiriamu Furaitaaku; Ishikawa, Eiji (du Pont de Nemours, E. I., and Co., USA). Jpn. Kokai Tokkyo Koho JP 61073067 A2 19860415 Showa, 13 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1985-201947 19850913.

AB Polymeric enzymes are bound to an antibody on its fragments to obtain a polymeric enzyme-antibody complex for immunoassay. As an example, .beta.-D-galactosidase in 0.05 M Tris-HCl buffer contg. 0.1k M NaCl,

0.0001 M MgCl₂ and 0.002 M EDTA (pH 6.5) was treated with an excess amt. of o-phenylenediimide to give polymeric .beta.-D-galactosidase, which was reacted with Fab'-SH (IgG fragment). In enzyme immunoassay, a sample contg. digoxin was incubated with a known amt. of the polymeric .beta.-D-galactosidase-Fab' complex prepd., the reaction mixt. was passed through a column contg. digoxin antibody-treated porous glass beads and free .beta.-galactosidase activity was colorimetrically measured for the calcn. of digoxin contents. Compared to a monomeric .beta.-galactosidase-Fab' complex, the sensitivity was markedly increased by using a polymeric .beta.-galactosidase-Fab' complex.

L23 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2003 ACS

1971:549349 Document No. 75:149349 Immunohistochemical localization of enterokinase in the porcine intestine. Takano, Kunio; Suzuki, Toshio; Yasuda, Kenjiro (Sch. Med., Keio Univ., Tokyo, Japan). Okajimas Folia Anatomica Japonica, 48(1), 15-87 (English) 1971. CODEN: OFAJAE. ISSN: 0030-154X.

AB The distribution of ***enterokinase*** was examd. in the porcine intestine by the fluorescent ***antibody*** method. The enzyme was concd. in the goblet cells and was diffusely located on the intestinal epithelium. This distribution pattern was quite different from the result obtained by staining the same tissue by the histochem. method for aminopeptidase.

L23 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2003 ACS

1946:37526 Document No. 40:37526 Original Reference No. 40:7255c-e Streptococcal fibrinolysis. I. The dissimilarity of serum protease and trypsin as indicated by the separate specificities of their kinases, fibrinolysin, and enterokinase. Kaplan, Melvin H. (Respiratory Dis. Commission, Reg. Station Hosp., Fort Bragg, NC). J. Clin. Investigation, 25, 331-6 (Unavailable) 1946.

AB The similarity between serum protease and trypsin with respect to their physicochem. properties and their physiol. effects has suggested their possible identity. Here evidence is presented that this is not the case. Fibrinolysin does not activate trypsinogen, nor is the serum protease activated by enterokinase. ***Antibodies*** to fibrinolysin do not inhibit the ***enterokinase*** of trypsinogen. Since the pertinent kinases are of different specific activities, it is unlikely that their enzymes should be identical.